Potential mycotoxin-producing species in organic cereals from Spain

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INTRODUCTION

By 2020, 10 % of Spanish agricultural area was intended for organic crop production and, in accordance with the 2030 Agenda, the goal is reaching 25 %. This important raise is due to the fact that organic farming is considered to be healthier and more environmentally friendly than conventional management. However, the agricultural practices that include the restriction on the use of chemical fungicides may facilitate the contamination of these crops by mycotoxigenic fungi. The presence of these species could cause a serious risk for human and animal health if environmental conditions were favorable for mycotoxin production.

Despite more than 215,000 hectares being intended for the cultivation of organic cereals, there are no reports regarding the occurrence of mycotoxin-producing molds in organic cereal crops in order to assess whether these agricultural procedures may affect the risk of mycotoxin presence. Cereals are often contaminated by *Aspergillus* and *Fusarium* genera, which produce aflatoxins, ochratoxin A (OTA), trichothecenes and fumonisins, mycotoxins classified as important carcinogens. Other common mold in cereals is *Penicillium brevicompactum*, which can produce mycophenolic acid, a potent immunosuppressant.

The aim of this study was to analyze the presence of the main mycotoxin-producing fungal species in Spanish organic cereals using species-specific PCR protocols.

MATERIALS & METHODS

A total of 83 cereal or pseudo-cereal samples (wheat, barley, spelt, oat, triticale, quinoa, teff and buckwheat) taken from the main Spanish cereal growing regions (Castilla y León, Castilla-La Mancha and Madrid) were examined.

The cereal grains were milled, and one gram was incubated in 50 mL of Sabouraud-Chloramphenicol broth (Condalab, Madrid, Spain) in darkness for 24 hours at 28ºC on an orbital shaker (130 rpm). Subsequently, the samples were filtered and grinded in liquid nitrogen using a mortar and a pestle prior to the isolation of total genomic DNA using the NZY Plant/Fungi gDNA Isolation kit (NZYTech, Lisbon, Portugal). DNA extraction was carried out in triplicate for each sample and DNA concentration was determined at 260 nm using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). The samples were preserved at -80 ºC until their analysis.

The main potential mycotoxin-producing fungal species were detected by nested PCR protocols. The fist step consists of a PCR using universal primers to amplify the ITS1-5.8S-ITS2 region, the elongation factor 1-alpha (EF-1α) and the partial calmodulin gene. The PCR products obtained were diluted (1:10) and species-specific PCR protocols were performed (Table 1). *Fusarium proliferatum*, *F. verticillioides* and *F. equiseti* were directly detected using species-specific PCR protocols without prior amplification. All PCR assays were performed in an Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany).

PCR products were detected in 1.25 % agarose gels in TAE 1X buffer (Tris Acetate 40 mM and EDTA 1.0 mM) including GreenSafe Premium (NZYTech, Lisbon, Portugal).

Table 1. PCR protocols.

Broad range PCR	Species-specific PCR	
ITS White et al., 1990	***A. flavus*** ◉	González-Salgado et al., 2008
	A. parasiticus ◉	Sardiñas et al., 2010
	A. carbonarius ★	Patiño et al., 2005
	A. steynii ★	Gil-Serna et al., 2009
	A. westerdijkiae ★	Gil-Serna et al., 2009
	P. brevicompactum ♦	Patiño et al., 2006
Calmodulin Peterson, 2008	***A. welwitschiae*** ★ ✚	Palumbo et al., 2014
	A. niger ★ ✚	Palumbo et al., 2014
EF-1α O'Donell et al., 1998	***F. langsethiae*** ▲	García-Díaz et al., 2020a
	F. sporotrichioides ▲	García-Díaz et al., 2020a
	F. poae ▲	García-Díaz et al., 2020a
	F. graminearum ♫ ☁	García-Díaz et al., 2020a
	F. temperatum ♣ ✚	García-Díaz et al., 2020a
	F. subglutinans ♣ ✚	Primers: Scauflaire et al., 2012. Conditions: García-Díaz et al., 2020a
	F. culmorum ♫ ☁	Jurado et al., 2005
	F. proliferatum ♣ ✚	Jurado et al., 2006
	F. verticillioides ♣ ✚	Patiño et al., 2004
	F. equiseti ♫ ▲	Jurado et al., 2005

Mycotoxins: ◉ Aflatoxins B1, B2, G1 y G2, ★ Ochratoxin A, ♣ Fumonisin B1, ✚ Fumonisin B2, ♫ Zearalenone ☁ Deoxynivalenol, ▲ Thricotecenes T-2 & HT-2, ♦ Mycophenolic acid

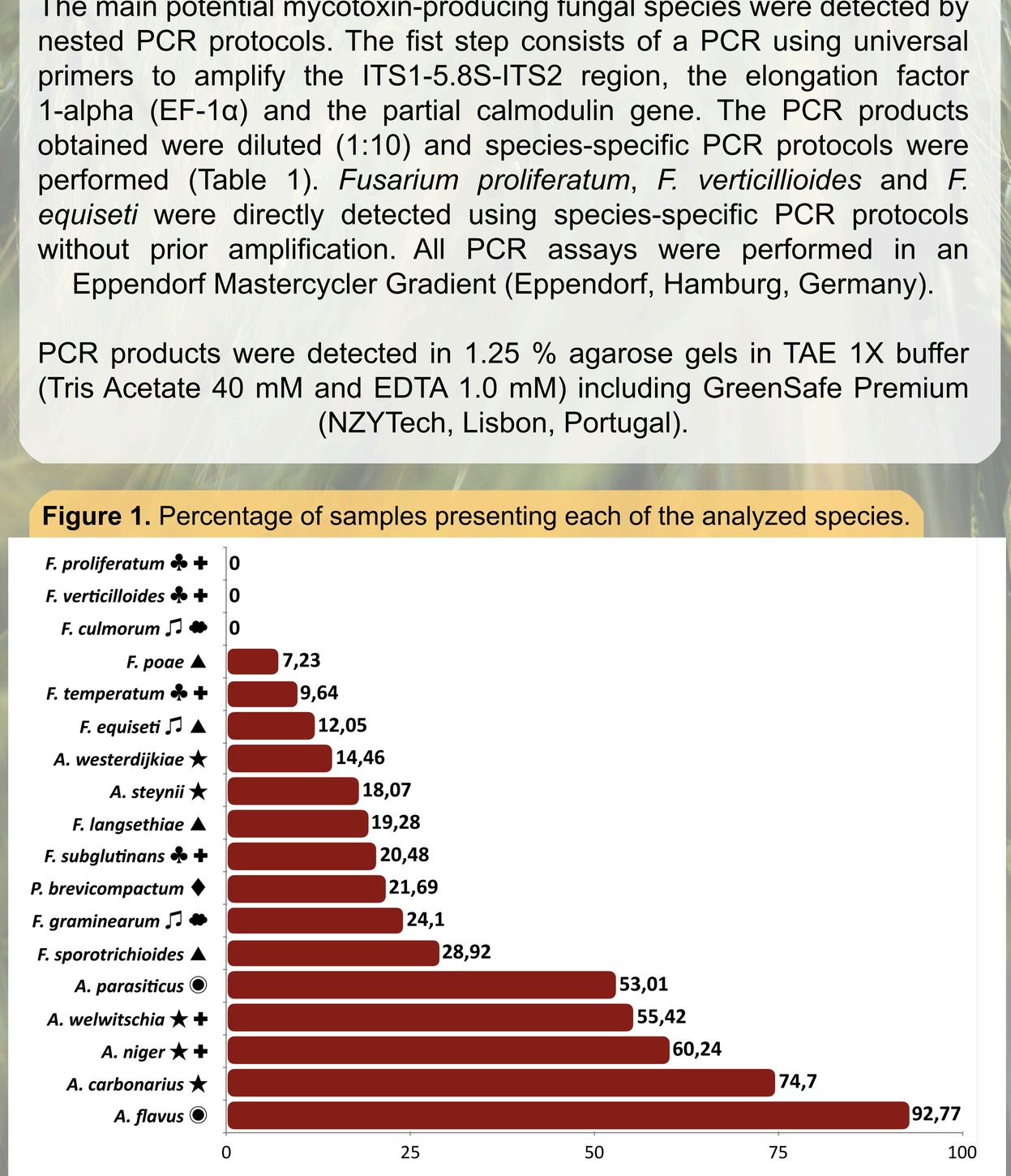
RESULTS AND DISCUSSION

The results obtained in this work confirm the contamination of organic cereals by mycotoxin-producing fungi. In all the samples analyzed, at least two mycotoxigenic species were detected.

The most frequently detected species was *Aspergillus flavus* (92 % of the samples) followed by *A. carbonarius* (75 %), *A. niger* (60 %), *A. welwitschiae* (54 %), and *A. parasiticus* (53 %).
Regarding *Fusarium* species, *F. graminearum*, *F. sporotrichioides* and *F. subglutinans* were found in a low percentage of samples (< 29 %) and in no case were *F. proliferatum*, *F. verticillioides* or *F. culmorum* detected in the organic cereals analyzed. *Penicillium brevicompactum* was found in 35 % of the samples (Fig. 1).

The presence of potentially mycotoxin-producing species in Spanish organic cereals might be indicating a high risk of aflatoxin and OTA contamination in the samples, as well as the co-occurrence of small levels of other mycotoxins such as trichothecenes. If environmental conditions (humidity, temperature) are favorable for fungal development and mycotoxin production, this could pose a serious health hazard.

Figure 1. Percentage of samples presenting each of the analyzed species.



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